Effects of thyrotoxicosis and selective hepatic autonomic denervation on hepatic glucose metabolism in rats

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Thyrotoxicosis is associated with a broad range of alterations in metabolism and energy homeostasis. Endogenous glucose production (EGP), lipolysis, and proteolysis are increased during thyrotoxicosis, providing the substrates needed for the concomitant increase in energy expenditure. Thyrotoxic patients exhibit increased EGP and hepatic insulin resistance, i.e., hampered suppressive action of insulin on EGP. It is widely assumed that the metabolic alterations during thyrotoxicosis represent direct effects of thyroid hormone (TH) on the expression of TH-responsive genes, mediated by binding of triiodothyronine (T3) to TH receptors in peripheral organs. Indeed, one of the main target organs for metabolic effects of TH is the liver, which has a key role in maintaining glucose homeostasis.

Until quite recently, the role of the central nervous system (CNS) as a focus of TH action was thought to be largely confined to development. However, evidence is accumulating that TH has many functions in the adult CNS as well, explaining, for example, the neurocognitive symptoms of hypothroidism and thyrotoxicosis. Recent studies have identified the autonomic nervous system (ANS), controlled by hypothalamic centers, as a key player in the regulation of glucose metabolism. We have previously demonstrated polysynaptic sympathetic and parasympathetic pathways between the hypothalamic paraventricular nucleus, known to control the autonomic efferent nerves, and the liver. Furthermore, we have shown that the hypothalamus can stimulate EGP via sympathetic input to the liver. The functional relevance of the parasympathetic input for hepatic glucose metabolism is evident from central effects of insulin and fatty acids on EGP that could be completely abolished by transection of the hepatic branch of the vagal nerve. Collectively, these observations indicate an important physiological role for both sympathetic and parasympathetic efferent branches of the ANS in regulating EGP.

It is presently unknown whether indirect effects of TH via the efferent branches of the ANS contribute to the changes in metabolism during thyrotoxicosis. We hypothesized that part of the changes in glucose metabolism during thyrotoxicosis are mediated via sympathetic or parasympathetic input to the liver. To test this hypothesis, we examined the effects of chronic, mild thyrotoxicosis on EGP, as well as hepatic insulin sensitivity, using euglycemic hyperinsulinemic clamps and stable isotope dilution in rats. We combined this with selective microsurgical sympathetic and parasympathetic hepatic denervations to study the role of these efferent ANS branches in the pathogenesis of thyrotoxicosis-induced changes in hepatic glucose metabolism.

MATERIALS AND METHODS

Animals

Male Wistar rats (Harlan, Horst, The Netherlands), housed under constant conditions of temperature (21 ± 1°C) and humidity (60 ± 2%) with a 12:12-h light-dark schedule (lights on at 7:00 AM), were used for all experiments. Animals were allowed to adapt to the new environment for 1 week before surgery. They were then divided into two groups: intact (I) and thyrotoxic (T).

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environment for ≥6 days before the first experimental manipulations. During adaptation, animals were housed in groups of four per cage. Body weight was between 325 and 375 g. Food and drinking water were available ad libitum. All of the following experiments were conducted with the approval of the Animal Care Committee of the Royal Netherlands Academy of Arts and Sciences.

Hormonal Treatment: Block and Replacement

On day 0 of the protocol, animals were placed in individual cages (25 × 25 × 35 cm) and treated with 0.025% methimazole (Sigma) in drinking water containing 0.3% saccharin. On day 7, osmotic minipumps (flow rate of 5 μl/h, Alzet 2ml2; Duract, Cupertino, CA) loaded with L-thyroxine (T4; Sigma) solved in 6.5 mM NaOH and 50% propylene glycol were implanted under the dorsal skin during the surgical procedure (see below). Osmotic minipumps delivered either 1.75 (replacement dose, euthyroid groups) or 16 μg (thyrotoxic groups) of T4 (100 g body wt/day).

Surgery

General procedure. On day 7, animals were anesthetized using a mixture of Hypnorm (0.07 ml/100 g body wt im; Janssen) and Dormicum (0.04 ml/100 g body wt sc; Roche). During abdominal surgery, the abdominal cavity was bathed regularly with saline to prevent drying of the viscera. The abdominal wall and skin were closed separately with sutures (5-0 Perma-Hand Said Ethicon). After surgery, the animals were placed in an incubator (temperature 30°C) until being awakened, and saline (10 ml) was injected subcutaneously to compensate for deficient fluid intake during recovery. Postoperative care was provided by subcutaneous injection of Temgesic (Utrecht importor; Schering-Plough), 0.01 ml/100 g body wt, on the morning after surgery.

Jugular vein and carotid artery cannulation. In all animals an intra-atrial silicone cannula was implanted through the right jugular vein for infusion (35), and a second silicone cannula was placed in the left carotid artery for blood sampling. Both cannulas were tunneled to the head subcutaneously, fixed with dental cement to four stainless steel screws insered into the skull. A mixture of 60% amoxicillin, 20% heparin, and 20% saline in polyvinlypyruvidon (Sigma) was used to fill the cannulas and prevent inflammation and occlusion. In the 10 days between surgery and the hyperinsulinemic clamps, this mixture was replaced at least three times.

Hepatic denervations. A laparotomy was performed in the midline. The liver lobes were gently pushed up and the ligaments around the liver lobes severed. For hepatic sympathectomy (Sx), the bile duct and the portal vein complex were visualized with an operating microscope (×25 magnification). The bile duct was isolated from the portal vein complex, and all tissue running along the bile duct was transected using microsurgical instruments. At the level of the hepatic portal vein, the hepatic artery divides into the hepatic artery proper and the gastroduodenal artery. This division occurs on the ventral surface of the portal vein. At this point, the arteries were separated via blunt dissection from the portal vein. All nerve bundles running along the hepatic artery proper were removed. Any connective tissue attachments between the hepatic artery and the portal vein were cut, eliminating any possible nerve crossings. The sympathetic denervation involves an impairment of both efferent and afferent nerves, but this procedure does not impair the parasympathetic vagal input to the liver, as shown previously (20).

For hepatic parasympathectomy (Px), the fascia containing the common hepatic vagal branch was stretched by gently moving the stomach, revealing the hepatic vagal branch as it separated from the stomach, revealing the hepatic vagal branch as it separated from the left vagal trunk. The neural tissue was transected between the ventral vagal trunk and the liver. The fascia between the stomach/ esophagus and the liver were also transected to remove any additional small branches (20).

Sham-operated rats, also referred to as intact (Int) rats, underwent all procedures as described above, except for transection of the neural tissue.

Hyperinsulinemic Euglycemic Clamp and Stable Isotope Dilution

On day 17, a hyperinsulinemic euglycemic clamp combined with stable isotope dilution was performed in all animals. Each experiment consisted of a tracer equilibration period (t = −75 to t = −15 min), a basal period (t = −15 to t = 0 min), and a hyperinsulinemic period (t = 0 to t = 160 min). The protocol was based on a validated hyperinsulinemic euglycemic clamping protocol for mice (37) that was adjusted for the use of stable isotope tracers and body weight.

In the afternoon on the day before the hyperinsulinemic clamp, rats were connected to a metal collar attached to polyethylene tubing (for blood sampling and infusion) that was kept out of reach of the animals by a counterbalanced beam. This allowed all subsequent manipulations to be performed outside the cages without the animals being handled. At 1600, a venous blood sample was obtained for determination of plasma concentrations of thyroid-stimulating hormone (TSH), T3, and T4.

On the hyperinsulinemic clamp day, food was removed from the cages 5 h before the first basal measurements. At 11:00 AM a primed (8.0 μmol in 5 min), continuous (16.6 μmol/h) infusion of the stable isotope tracer [6,6-2H2]glucose (>99% enriched; Cambridge Isotope Laboratories, Cambridge, MA) was started using an infusion pump (Harvard Apparatus, Holliston, MA). Before this, a blood sample for determination of background isotopic enrichment was taken (200 μl, t = −75 min). After 60 min of equilibration time, blood samples (200 μl) were obtained for measurement of glucose concentration, isotopic enrichment (t = −15, −5, and 0 min), and plasma insulin concentration (t = −15 min).

Subsequently, a primed (10 mU in 4 min) followed by continuous (62.5 mU/h) infusion of human recombinant insulin (Actrapid 100 IU/ml; Novo Nordisk, Alphen aan de Rijn, The Netherlands) was started to maintain euglycemia at 5.5 mmol/l (clamping period), 25% glucose was infused at a variable rate. Blood glucose concentrations were measured every 10 min, and consequently, glucose infusion rate was adjusted if needed. The 25% glucose solution was 1% enriched with [6,6-2H2]glucose to approximate the values for enrichment reached in plasma, thereby minimizing changes in isotopic enrichment due to variable infusion rates of exogenous glucose. At t = 100 min, blood samples (200 μl) were obtained for measurement of plasma glucose concentration, isoepthene enrichment (t = 100, 115, 130, 145, and 160 min), and plasma insulin concentration (t = 100 min). After the clamp, rats were killed, and liver tissue was snap-frozen and stored at −80°C for subsequent analysis. EGP and rate of disappearance (Ra) were calculated using modified forms of Steele equations (14, 34).

Plasma Measurements

Plasma glucose concentrations were determined in blood spots (<5 μl) using a glucose meter (Freestyle, Abbott, The Netherlands), with inter- and intra-assay coefficients of variation (CV) of <6 and 4%, respectively. Plasma concentrations of T3 and T4 were determined by an in-house RIA (19), with inter- and intra-assay CV of 7–8 and 3–4% (T3) and 3–6 and 2–4% (T4), respectively. Detection limits for T3 and T4 were 0.3 and 5 mmol/l, respectively. Plasma TSH concentrations were determined by a chemiluminescent immunoassay (Immulite 2000; Diagnostic Products, Los Angeles, CA), using a rat-specific standard. The inter- and intra-assay CV for TSH were <4 and 2% at >3.5 mU/l, respectively, and the detection limit was 0.60 mU/l. Plasma insulin was measured by a commercially available ELISA (Mercodia, Uppsala, Sweden). The inter- and intra-assay CV for TSH were 4 and 2%, and the detection limit was 13 pmol/l. Glucose enrichment was measured as described previously (1). The [6,6-2H2]glucose enrichment (tracer/tracee ratio) interassay CV was 1%, the intra-assay CV 1%, and the detection limit 0.04%.
HPLC Electrochemical Measurements

To check the effectiveness of the hepatic Sx, norepinephrine (NE) content in the liver was measured. Liver tissue samples of 50 mg were homogenized in 1 ml of ice-cold NH4Cl buffer (0.2 M, containing 12 nM/1 α-methylnorepinephrine and 1 g/l EDTA, pH 7.0) and centrifuged twice (14,000 rpm) for 15 min at 4°C. NE was determined with an in-house HPLC method. Essentially, NE was selectively isolated by liquid-liquid extraction (32) and derivatized with the fluorescent 1,2-diphenylethylenediamine (38). The fluorescent derivatives were separated by reversed-phase liquid chromatography and detected by scanning fluorescence detection (510 pump, 717 plus autosampler, and 474 scanning fluorescence detector; Waters Chromatography).

Separation of NE from other endogenous compounds was achieved with a Waters Xterra RP18 column (5 μm, 3.9 × 150 mm). As an internal standard, α-methylnorepinephrine was used. Intra- and inter-assay CV were 3 and 20%, respectively. The detection limit for NE was 0.05 nmol/l.

We (20) have previously evidenced the effectiveness of our method for selective hepatic parasympathectomy by using retrograde viral tracing. In these studies, the success rate of hepatic parasympathectomy was >90%.

RNA Isolation and Real-Time PCR

mRNA was isolated from 10 mg of liver tissue using a Magna Pure apparatus and a Magna Pure LC mRNA isolation kit II (tissue; Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer’s protocol. cDNA synthesis was performed with the first-strand cDNA synthesis kit for RT-PCR (AMV; Roche Molecular Biochemicals). Previously published primer pairs (36) were used to amplify hypoxanthine phosphoribosyl transferase (HPRT; a housekeeping gene). We designed primer pairs for phosphoenolpyruvate carboxykinase (PEPCK) and type 1 iodothyronine deiodinase (D1) with the following sequences: PEPCK forward TGCCCTCTCCCCT-TAAAAGAG and reverse CGCTCCGAAGGATGATCT, D1 forward GAAGTGCAAGTCTGGAATT and reverse CTCGCC-GAAGTTCAACACCA. Real-time PCR was performed using the LightCycler (Roche Molecular Biochemicals) as described earlier (4). PCR programs were as follows: predenaturation for 10 min at 95°C; amplification for 45 cycles, which consists of denaturation for 10 s at 95°C; annealing at various temperatures for 10 s; and elongation for 15 s at 72°C (annealing temperature: HPRT 54°C, PEPCK 55°C, and D1 55°C). For quantification, standard curves were generated of a sequence-specific PCR product ranging from 0.01 fg/μl to 100 fg/μl. Samples were corrected according to their mRNA content using HPRT mRNA and plotted as relative expression. Samples were individually checked for their PCR efficiency (29). The median of the efficiency was calculated for each assay, and samples with a >0.05 difference of the efficiency median value were excluded from the analysis.

Statistics

Data were analyzed by mixed-model analysis of variance (ANOVA), with nature of denervation (Int, Sx, Px) and thyroid hormonal status (euthyroid, thyrotoxic) as fixed effects. Significance was defined at P < 0.05. A least significant difference (LSD) post hoc test was performed if ANOVA revealed significance to determine which experimental groups differed from each other. Student’s one-sample t-test was used to determine statistical differences from zero. A Mann-Whitney U-test was used to analyze the PCR data. Pearson correlation was used to test for associations between factors. Data are presented as means ± SE.

RESULTS

Six groups of rats were studied, i.e., three euthyroid and three thyrotoxic groups. Euthyroid rats were either sham operated (Eu Int, n = 10) or underwent a selective hepatic sympathetic (Eu Sx, n = 7) or parasympathetic (Eu Px, n = 9) denervation. The same applied to thyrotoxic rats (Tox Int, n = 10; Tox Sx, n = 7; Tox Px, n = 10).Selective denervation of the sympathetic input to the liver resulted in a significant 72% reduction in hepatic NE content [13.6 ± 2.3 vs. 49.1 ± 4.5 ng/g liver, P < 0.01, Sx (n = 14) vs. Int (n = 20), respectively]. On the other hand, the hepatic NE content of animals with a selective parasympathetic denervation did not differ from intact animals [47.1 ± 8.6 ng/g liver, Px (n = 19)].

Body Weight and Eating Behavior

At the time of surgery, there was no difference in body weight between groups (Eu Int 348 ± 5, Eu Sx 344 ± 4, Eu Px 358 ± 4, Tox Int 353 ± 8, Tox Sx 350 ± 7, Tox Px 362 ± 5 g; not significant).

The changes in body weight after surgery and the overnight food intake before the clamp for each experimental group are depicted in Fig. 1. As expected, all thyrotoxic groups lost weight between surgery and the hyperinsulinemic clamp, in contrast to euthyroid groups. Nevertheless, food intake was higher (P < 0.01) in all thyrotoxic groups compared with Eu Int rats, which is in accordance with increased energy expenditure during thyrotoxicosis. It is important to note that body weight increased in all groups during the 3 days preceding the clamp, indicating full recovery from surgery and a positive energy balance.

Plasma Thyroid Hormones

Plasma thyroid hormone concentrations at the time of the hyperinsulinemic clamps after 10 days of T4 treatment are depicted in Table 1.

In Eu Int rats, biochemical euthyroidism was evidenced by TSH concentrations similar to those reported earlier (18, 19) in control rats without hormonal treatment. Plasma TSH concentrations were between 2.00 and 3.00 mU/l in all euthyroid groups. TSH was suppressed to levels below the limit of detection in all thyrotoxic animals. Plasma T3 and T4 concentrations were significantly higher in Tox Int compared with Eu Int rats (4.3-fold, P < 0.01, and 2.0-fold, P < 0.01, respectively). In euthyroid rats, hepatic autonomic denervation did not affect plasma T3 and T4. However, in thyrotoxic rats both sympathetic and parasympathetic hepatic denervation lowered plasma T3 and T4, but not the T3/T4 ratio, compared with intact animals. In Tox Int rats, the T3/T4 ratio was significantly increased compared with Eu Int animals.

Glucose, Insulin, Glucose Kinetics, and Hepatic mRNA Expression

Basal state. Tox Int rats exhibited increased basal plasma glucose concentration compared with Eu Int rats (Fig. 2A). Basal insulin concentration was not affected by thyrotoxicosis per se (Fig. 2B). In accordance with the increase in basal plasma glucose concentration, basal EGP was increased by 45% in Tox Int compared with Eu Int rats (Fig. 2C). In line with this, mRNA expression of PEPCK, the rate-limiting enzyme of gluconeogenesis, was increased 3.5-fold in thyrotoxic animals (Fig. 3A). In addition, mRNA expression of the T3 responsive gene deiodinase type 1 was upregulated fourfold
The primary findings of this study are that chronic, mild thyrotoxicosis in rats increases EGP, whereas it decreases relative suppression of EGP during hyperinsulinemic clamps, indicating hepatic insulin resistance. This is supported by a highly significant positive correlation between plasma T3 and EGP. The increased EGP during thyrotoxicosis can be attenuated by selective hepatic Sx. Selective hepatic Px increases plasma insulin in thyrotoxic rats without a change in EGP, indicating hepatic insulin resistance. These combined findings indicate that T3 is an important direct determinant of EGP in thyrotoxicosis with a small contribution via the sympathetic nervous system. Furthermore, parasympathetic innervation of the liver may function to restrain EGP during mild thyrotoxicosis, because after Px more insulin is needed to keep EGP at the level found in mild thyrotoxicosis.

**DISCUSSION**

In euthyroid rats, selective hepatic sympathetic or parasympathetic denervation did not affect glucose concentration, insulin concentration, or EGP. In thyrotoxic rats, there was no statistically significant effect of sympathetic denervation on basal EGP in thyrotoxic animals; however, the relative increase of EGP in Tox Sx rats compared with Eu Int rats was smaller than in the other thyrotoxic groups (Fig. 2C). In line with this, Tox Sx animals exhibited unaltered glucose concentration compared with Eu Int rats, in contrast to the increased glucose concentration in Tox Int and Tox Px rats (Fig. 2A). During thyrotoxicosis, selective hepatic Px induced a marked increase in basal insulin concentration (Fig. 2B). This increase in insulin concentration was accompanied by unaltered glucose concentration and EGP in Tox Px compared with Tox Int animals, indicating hepatic insulin resistance. There was no effect of hepatic denervation in both euthyroid and thyrotoxic rats on hepatic mRNA expression of PEPCK and D1 (data not shown).

**Hyperinsulinemic state.** After 100 min of insulin infusion, insulin concentrations increased in all groups \((P < 0.05)\) compared with the basal state (mean increment 229 ± 25 pmol/l). There was no difference in plasma insulin concentration between groups. As expected, EGP decreased in all groups in response to hyperinsulinaemia. During the clamp, EGP was 122% higher in Tox Int compared with Eu Int rats. There was no effect of sympathetic denervation on hyperinsulinemic EGP in euthyroid or thyrotoxic rats. Parasympathetic denervation slightly increased hyperinsulinemic EGP in euthyroid and thyrotoxic rats, although this effect missed statistical significance (Fig. 4A). In Tox Int rats, the relative suppression of EGP in the hyperinsulinemic compared with the basal state (%suppression of EGP) was decreased relative to Eu Int rats (Fig. 4B). There was a highly significant positive correlation between plasma T3 concentration and EGP in both the basal and hyperinsulinemic states (Fig. 5). \(R_d\) was similar between the groups (data not shown).

**Table 1.** \(T_3, T_4,\) and TSH plasma concentration at the time of the hyperinsulinemic clamps in experimental animals

<table>
<thead>
<tr>
<th></th>
<th>Eu Int</th>
<th>Eu Sx</th>
<th>Eu Px</th>
<th>Tox Int</th>
<th>Tox Sx</th>
<th>Tox Px</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>10</td>
<td>7</td>
<td>9</td>
<td>10</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>(T_3, \text{nmol/l})</td>
<td>0.86±0.08</td>
<td>0.88±0.05</td>
<td>0.73±0.04</td>
<td>3.66±0.31**</td>
<td>2.78±0.33***</td>
<td>2.81±0.18***</td>
</tr>
<tr>
<td>(T_4, \text{nmol/l})</td>
<td>130±6</td>
<td>111±9</td>
<td>135±5</td>
<td>255±18**</td>
<td>169±15**</td>
<td>209±17**</td>
</tr>
<tr>
<td>TSH, mU/l</td>
<td>2.53±0.94</td>
<td>2.13±1.02</td>
<td>2.85±0.83</td>
<td>&lt;0.60*</td>
<td>&lt;0.60*</td>
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Data are means ± SE. \(T_3,\) triiodothyronine; \(T_4,\) L-thyroxine; Eu Int, euthyroid sham liver denervated; Eu Sx, euthyroid hepatic sympatectomized; Eu Px, euthyroid hepatic parasympatectomized; Tox Int, thyrotoxic sham liver denervated; Tox Sx, thyrotoxic hepatic sympatectomized; Tox Px, thyrotoxic hepatic parasympatectomized. \(*P < 0.05\); \;++P < 0.01 vs. Eu Int; \;++P < 0.01 vs. Tox Int as revealed by post hoc least significant difference test only when ANOVA indicated \(P < 0.05\) for the respective factor.

**Fig. 1.** A: food consumed by euthyroid (left) and thyrotoxic (right) groups the night before the hyperinsulinemic clamp. ANOVA was significant \((P < 0.0001)\) for factor thyroid hormone status. \;++P < 0.01 vs. euthyroid sham liver-denervated (Eu Int) rats as revealed by post hoc least significant difference (LSD) test. B: body weight difference \((\Delta BW)\) between time of surgery and time of the hyperinsulinemic clamp in experimental groups. Note that, despite the increase in food consumption \((A)\), none of the thyrotoxic groups regained their time-of-surgery body weight at the time of the hyperinsulinemic clamps. \#P < 0.01; \(H_0: \Delta BW = 0\), as revealed by Student’s 1-sample \(t\)-test. Data are means ± SE. No. of animals/experimental group is depicted under the bars in A. Eu Sx, euthyroid hepatic sympatectomized; Eu Px, euthyroid hepatic parasympatectomized; Tox Int, thyrotoxic sham liver denervated; Tox Sx, thyrotoxic hepatic sympatectomized; Tox Px, thyrotoxic hepatic parasympatectomized.
The striking resemblance between many of the effects of thyrotoxicosis and sympathetic nervous stimulation has long been noted (23). Because of this similarity, the syndrome of goiter, exophthalmus, and tachycardia (i.e., the Merseberg triad) was regarded a disease of the sympathetic nervous system by physiologists at the time. By the end of the 19th century, this led to the surgical treatment of severe thyrotoxicosis by cervical sympathetic chain resection (28) and later by high spinal anesthesia or adrenal denervation (10). These practices were gradually abandoned with increasing knowledge of the thyroid gland and of TH. However, it is still common practice nowadays to start treatment of severe thyrotoxicosis with β-adrenergic blockers until a clinical effect of antithyroid drugs is reached.

In the literature, the idea of increased sympathetic tone during hyperthyroidism has gradually moved to the background. However, as more accurate techniques for measuring sympathetic tone have become available, evidence is building up for increased sympathetic neural output to white adipose tissue in hyperthyroid patients (16). In addition, hyperthyroid patients exhibit increased sympathetic and decreased parasympathetic output to the heart, as revealed by heart rate spectral analysis (5, 7).

To discriminate between peripheral and central effects of TH, an experimental model is needed in which central and peripheral manipulations can be performed without the systemic TH milieu being interfered with. For this, we have used “block and replacement” treatment in rats. This means that rats are treated with the thyreostatic methimazole in drinking water to inhibit TH synthesis, and simultaneously, T4 is administered by use of osmotic minipumps. In this way, T4 is released continuously, mimicking the release of TH by the thyroid gland. We used two doses of T4, i.e., a replacement dose giving rise to sustained euthyroidism and a ninefold higher dose of T4.

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inducing mild thyrotoxicosis. The duration of T4 administration was 10 days, resulting in a chronic state of mild thyrotoxicosis in rats treated with the highest T4 dose, as evidenced by a 4.3-fold and a 2.0-fold increase in plasma T3 and T4, respectively, and a decrease in plasma TSH. In line with this, the hepatic mRNA expression of D1, a T3-responsive gene that is a sensitive marker of peripheral thyroid status (40), showed a fourfold increase in thyrotoxic animals.

In the present study, we have shown for the first time that mild thyrotoxicosis induces hepatic insulin resistance in freely moving, conscious rats. A hyperinsulinemic euglycemic clamp combined with isotope dilution is the gold standard for measuring hepatic insulin sensitivity (13). Although there have been reports of altered EGP during thyroid hormone excess in vivo (17, 26), to our knowledge, until now hyperinsulinemic euglycemic clamps combined with isotope dilution have never been used to study alterations in EGP and its sensitivity to insulin in thyrotoxic rats.

In the literature, data on the effect of hepatic Px on EGP and hepatic insulin resistance are discordant. Studies combining manipulation of fatty acid metabolism and hepatic vagal denervation in rats (22, 27) point to an important role of the hepatic vagal nerve in mediating the effects of central lipid sensing on EGP. Likewise, the repressive effect of intracerebroventricularly infused insulin on EGP can be completely abolished by hepatic vagal denervation (25). In these and other studies (3), hepatic Px in itself does not affect EGP. In the present study, during thyrotoxicosis but not during euthyroidism, selective hepatic Px induced insulin resistance. The notion arises that a consistent effect of vagal hepatic denervation becomes manifest only in combination with an additional stimulus such as manipulation of central lipid sensing, intracerebroventricular insulin infusion, or systemic thyroid hormone excess.

We observed a decrease in plasma concentrations of T3 and T4 by both Sx and Px in thyrotoxic rats, although equal doses of T4 were administered via osmotic minipumps in thyrotoxic-intact and thyrotoxic-denervated animals. Hepatic denervation did not result in significant changes in hepatic D1 mRNA expression, in accordance with the unaffected T3/T4 ratio. Thus, altered synthesis or clearance of TH-binding proteins such as transthyretin in the liver or altered hepatic clearance of TH resulting from hepatic autonomic denervation during thyrotoxicosis is a more plausible explanation.

The present study shows that the alterations in glucose metabolism induced by thyrotoxicosis are slightly modulated by selective hepatic autonomic denervation. Thus, the efferent autonomic nerves may be responsible for part of these changes. At this stage, it remains unknown which CNS areas control the ANS efferent nerves affecting hepatic glucose metabolism during thyrotoxicosis. The hypothalamus is a key central site of TH action, and both the human (2) and rat (24) hypothalamus abundantly express TH receptors. This specifically applies to

Fig. 4. A: EGP during the hyperinsulinemic clamp of experimental groups. Note increased EGP in all thyrotoxic groups relative to Eu rats. ANOVA was significant (P < 0.0001) for factor thyroid hormonal status. ^^^P ≤ 0.01 vs. Eu Int as revealed by post hoc LSD test. B: %suppression of EGP in the hyperinsulinemic state relative to the basal state of experimental groups. Note that all thyrotoxic groups exhibit decreased %suppression of EGP compared with Eu Int rats, indicating hepatic insulin resistance during thyrotoxicosis. ANOVA indicated P < 0.009 for factor thyroid hormonal status, P = 0.216 for factor denervation. ^P ≤ 0.05 vs. Eu Int as revealed by post hoc LSD test. Data are means ± SE. No. of animals/experimental group is depicted under the bars in A.

Fig. 5. Relation between basal EGP (A) or hyperinsulinemic EGP (B) and plasma triiodothyronine (T3) concentration in all experimental animals. Pearson correlation coefficient and P values are depicted in A and B, bottom right.
the paraventricular nucleus, where the (pre)autonomic neurons that control sympathetic and parasympathetic motor neurons are located, and the arcuate nucleus, where the blood-brain barrier is absent. This suggests that the hypothalamus is perfectly equipped to sense and process TH signals, not only via the neuroendocrine route but also via its connections with the ANS, and to regulate hepatic glucose metabolism. The observation that other hormones such as insulin (25), estrogen (8), and glucocorticoids (11) affect metabolism via central (hypothalamic) sites of action independently of the peripheral hormonal milieu supports the possibility that TH may affect autonomic outflow from the hypothalamus to the liver. However, we are aware of only one study addressing peripheral physiological effects of centrally administered TH. In that study (15), an intracerebroventricular bolus infusion of T₃ in surgically thyroidectomized hypothyroid rats increased heart rate. The same dose showed no effect after intravenous infusion, suggesting T₃-responsive CNS regulation of heart rate. T₃ is an important direct determinant of EGP upon central administration in physiological euthyroid conditions.

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